FUNCTIONAL AND CRYPTOREGIO-CHEMICAL STUDY OF desC GENE ENCODING Δ⁹ FATTY ACID DESATURASE FROM Spirulina platensis C1 (Arthrospira sp. PCC 9438)

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A gene encoding for the Δ⁹ desaturase was cloned from genomic library of cyanobacterium S. platensis C1 (Arthrospira sp. PCC 9438). The deduced amino acid sequence of this desaturase exhibits high homology with the cloned Δ⁹ desaturases from other organisms. Moreover, it contains three-conserved histidine boxes and two hydrophobic domains (characteristic of desaturases) which are responsible for catalytic function and membrane spanning, respectively. Consequently, the functional analysis of this enzyme was confirmed by complementation with mutant yeast lacking Δ⁹ desaturase activity. The accumulation of oleic acid in the transformed mutant yeast but not in the control indicates that the S. platensis C1 desC gene encodes for a protein with Δ⁹ desaturase activity. Moreover the cryptoregiochemistry of this enzyme was also characterized in search for initial oxidation site in fatty acid chain. This has been investigated through the use of kinetic isotope effect experiments with deuterated substrates. The primary deuterium kinetic isotope effects (KIEs) were performed by culturing yeast transformants with deuterated thia-substituted stearic acids, A large kinetic isotope effect was found for the 9 position, in qualitative agreement with results from eukaryotic desaturases. This result indicates that Δ⁹ desaturation is initiated by an energetically C-H bond cleavage at the carbon closer to the carboxyl terminus.